Sequential or Combination Antifungal Therapy with Voriconazole and Liposomal Amphotericin B in a Guinea Pig Model of Invasive Aspergillosis

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We evaluated combinations of voriconazole (VRC) and liposomal amphotericin B (L-AMB) in a guinea pig invasive aspergillosis model. Simultaneous VRC and L-AMB was most effective, although VRC monotherapy was also effective. These regimens as well as sequential L-AMB followed by VRC were more effective than L-AMB alone or VRC followed by L-AMB.

Antifungal agents with distinct mechanisms of action offer the possibility of synergistic activity against invasive moulds when used in combination (1, 7, 8). The possibility of antagonism is raised when these drugs are given in combination, particularly sequentially, with drugs blocking ergosterol synthesis given before the polyenes (5, 11, 12).

We evaluated sequential and combination regimens with voriconazole (VRC) and liposomal amphotericin B (L-AMB) in a lethal immunosuppressed, transiently neutropenic guinea pig model of invasive aspergillosis. Male Hartley guinea pigs (0.5 kg) were immunocompromised and challenged with Aspergillus fumigatus as previously described (2). One day after cyclophosphamide administration, guinea pigs were challenged intravenously through the saphenous vein with 106 A. fumigatus conidia. Extensive infection develops throughout the liver, kidney, lung, and brain in this model, analogous to clinically disseminated invasive aspergillosis (6, 9–11). All animal research procedures were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio, TX.

Antifungal therapy included L-AMB (AmBisome, Fujisawa Healthcare, Inc., Deerfield, IL) and/or VRC (Pfizer, Inc., Groton, CT) initiated 24 h after challenge and continued for 5 days. L-AMB was diluted with sterile water to 3.0 mg/ml and was given intraperitoneally at 3 mg/kg/day. VRC was suspended in polyethylene glycol 200 (Sigma Chemical, St. Louis, MO) to 10 mg/ml and administered orally at 5 mg/kg twice a day (b.i.d.). Six groups of eight guinea pigs receiving the following treatments were included in this study: (i) untreated controls, (ii) L-AMB at 3 mg/kg/day, (iii) VRC at 5 mg/kg b.i.d., (iv) VRC at 5 mg/kg b.i.d. for 2 days followed by L-AMB at 3 mg/kg/day for 3 days (VRC→L-AMB), (v) L-AMB at 3 mg/kg/day for 2 days followed by VRC at 5 mg/kg b.i.d. for 3 days (L-AMB→VRC), and (vi) VRC at 5 mg/kg b.i.d. plus L-AMB at 3 mg/kg/day (VRC+L-AMB).

Organ cultures of brain, kidney, liver, and lung were performed postmortem (after the death of the animal during treatment [n = 35] or 96 h after completion of therapy for the remaining treated guinea pigs [n = 13]) (2–4).

Aspergillus fumigatus isolate P171, a clinical isolate that we used previously, was grown on Sabouraud dextrose slants at 37°C for 24 h and prepared for injection at 106 conidia/ml by hemacytometer count (2–4).

The Fisher exact test and the Wilcoxon rank sum test were used where appropriate. Statistical significance was defined as a P value of <0.05, adjusted for multiple-dose comparisons for each organ evaluated so that the level required for statistical significance was a P value of <0.0009.

Antifungal therapy was initiated 24 h after infection; untreated controls had a mean survival of 4.50 ± 0.27 days (range, 4 to 6 days) after challenge. The mortality and mean duration of survival of animals treated with VRC and L-AMB, each alone or in sequential use or in combination therapy along with untreated controls, are shown in Table 1 and Fig. 1. All treated animals had an increased duration of survival, ranging from an increase of almost 2 days (L-AMB alone) to 2 to over 3 days (all other treatment groups) compared to untreated controls, although statistical significance was achieved only in groups treated with VRC alone, L-AMB→VRC, and VRC+L-AMB (P < 0.0009). Death occurred in all eight untreated control guinea pigs, in 7/8 (88%) treated with L-AMB alone, and in 5/8 (63%) treated with VRC→L-AMB, com-

TABLE 1. Mean days of survival in temporarily immunosuppressed guinea pigs

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Mortality (% of total)</th>
<th>Survival (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE Range</td>
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<tr>
<td>Control (8)</td>
<td>8/8 (100)</td>
<td>4.50 ± 0.27</td>
</tr>
<tr>
<td>L-AMB (8)</td>
<td>7/8 (88)</td>
<td>6.13 ± 0.45</td>
</tr>
<tr>
<td>VRC (8)</td>
<td>1/8 (13)*</td>
<td>7.75 ± 0.25</td>
</tr>
<tr>
<td>VRC→L-AMB (8)</td>
<td>5/8 (63)</td>
<td>6.75 ± 0.41</td>
</tr>
<tr>
<td>L-AMB→VRC (8)</td>
<td>1/8 (13)*</td>
<td>7.88 ± 0.13</td>
</tr>
<tr>
<td>VRC+L-AMB (8)</td>
<td>1/8 (13)*</td>
<td>7.88 ± 0.13</td>
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a Survival from day of challenge with Aspergillus fumigatus.

b P < 0.0009 versus controls.
pared to mortality in 1/8 (13%) animals treated with either VRC, L-AMB→VRC, or VRC+L-AMB (P < 0.0009 versus controls), as shown in Table 1.

Results of semiquantitative organ cultures are shown in Table 2. Control animals showed extensive infection in the tissues examined. All treatment groups had reduced tissue burdens in one or more cultured organs compared to controls, although this reduction achieved statistical significance only in groups treated with VRC alone and VRC+L-AMB. Both VRC alone and VRC+L-AMB were highly effective at reducing the tissue burden in all organs cultured, including the brain (P < 0.0009 versus controls), with overall lower values with the combination regimen (reductions ranging from −1 to >3 log CFU/g versus controls). Compared to sequential VRC→L-AMB, sequential L-AMB→VRC yielded greater reductions in tissue burdens in liver, lung, and kidney but not brain (not significant [NS]). L-AMB alone and VRC→L-AMB were the least effective regimens in reducing fungal tissue burden. L-AMB alone reduced liver, kidney, and brain burdens by 1 to 2 log CFU/g versus controls (NS), while VRC→L-AMB reduced liver burden by more than 1 log CFU/g, but with only minimal reductions in the other organs. The sequential regimen of L-AMB→VRC produced lower counts in liver and lung than L-AMB alone (NS) but produced similar counts in kidney and brain.

Numbers of positive organ cultures from treated animals and untreated controls are also shown in Table 2. All tissues cultured from untreated controls were positive for Aspergillus. Results of treatments with L-AMB and VRC→L-AMB were similar to those for controls; neither regimen effectively sterilized tissues at the doses tested, with organ tissue cultures positive in 31/32 and 30/32 organs tested, respectively. Fewer positive cultures were seen with either VRC (21/32 positive organ cultures) or L-AMB→VRC (20/32 positive cultures) than with L-AMB alone or VRC→L-AMB (P < 0.009). Overall, treatment with the combination VRC+L-AMB was more effective in reducing positive cultures in liver, lung, and kidney tissues than were any of the other therapeutic regimens compared to controls; two of eight (25%) animals

### Table 2. Semiquantitative organ cultures of temporarily immunosuppressed guinea pigs treated with antifungal therapy begun 24 h after challenge and sacrificed 96 h after completion of therapy

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Liver</th>
<th>Lung</th>
<th>Kidney</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>3.26 ± 0.28 (8/8)</td>
<td>1.46 ± 0.28 (8/8)</td>
<td>3.11 ± 0.09 (8/8)</td>
<td>2.93 ± 0.15 (8/8)</td>
</tr>
<tr>
<td>L-AMB (8)</td>
<td>1.10 ± 0.44 (8/8)</td>
<td>0.71 ± 0.28 (7/8)</td>
<td>1.67 ± 0.42 (8/8)</td>
<td>1.84 ± 0.48 (8/8)</td>
</tr>
<tr>
<td>VRC (8)</td>
<td>0.53 ± 0.29* (4/8)</td>
<td>0.54 ± 0.20 (7/8)</td>
<td>1.21 ± 0.27† (7/8)</td>
<td>0.16 ± 0.13* (3/8)</td>
</tr>
<tr>
<td>VRC→L-AMB (8)</td>
<td>1.94 ± 0.35 (8/8)</td>
<td>1.21 ± 0.26 (7/8)</td>
<td>2.96 ± 0.20 (8/8)</td>
<td>2.44 ± 0.38 (7/8)</td>
</tr>
<tr>
<td>L-AMB→VRC (8)</td>
<td>0.45 ± 0.41 (2/8)</td>
<td>0.52 ± 0.31 (3/8)</td>
<td>1.95 ± 0.46 (7/8)</td>
<td>2.35 ± 0.27 (8/8)</td>
</tr>
<tr>
<td>VRC+L-AMB (8)</td>
<td>0.0* (0/8)</td>
<td>0.16 ± 0.13* (3/8)</td>
<td>0.88 ± 0.36* (5/8)</td>
<td>0.94 ± 0.47 (4/8)</td>
</tr>
</tbody>
</table>

*P < 0.0009 versus controls; †P < 0.0009 versus VRC→L-AMB.
yielded all sterile cultures, and in this group, only 12 of 32 cultures were positive for \textit{Aspergillus} \((P < 0.0009\) versus controls, L-AMB, or VRC→L-AMB).

The VRC+L-AMB combination was most effective at reducing tissue burden, sterilizing tissues, and reducing mortality, although VRC monotherapy was also effective. These two regimens as well as the sequential use of L-AMB→VRC were more effective than L-AMB alone or VRC→L-AMB. An increased length of survival and decreased overall mortality were seen with all the treatment regimens. This suggests that frank antagonism did not occur with either sequential regimen or with combination therapy. The combination of these two drug classes could be beneficial and should be further evaluated for treating patients with this often lethal infection.

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REFERENCES


